Plant-Parasitic Nematodes Are Potential Pathogens of *Miscanthus* × *giganteus* and *Panicum virgatum* Used for Biofuels

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Abstract

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A survey of *Miscanthus* × *giganteus* and switchgrass plots throughout the midwestern and southeastern United States was conducted to determine the occurrence and distribution of plant-parasitic nematodes associated with these biofuel crops. During 2008, rhizosphere soil samples were collected from 24 *Miscanthus* × *giganteus* and 38 switchgrass plots in South Dakota, Iowa, and Illinois. Additional samples were collected from 11 *Miscanthus* × *giganteus* and 10 switchgrass plots in Illinois, Kentucky, Georgia, and Tennessee the following year. The 11 dominant genera recovered from the samples were *Pratylenchus*, *Helicotylenchus*, *Xiphinema*, *Longidorus*, *Heterodera*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconemella*, *Paratrichodorus*, *Hemicriconemoides*, and *Paratylenchus*. Populations of *Helicotylenchus*, *Xiphinema*, and *Pratylenchus* were common and recorded in 90.5, 83.8, and 91.9% of the soil samples from *Miscanthus* × *giganteus*, respectively, and in 91.6, 75, and 83.3% of the soil samples from switchgrass, respectively. Prominence value (PV) (PV = population density × $\sqrt{\text{frequency of occurrence}/10}$) was calculated for the nematodes identi-

fied. *Helicotylenchus* had the highest PV (PV = 384) and was followed by *Xiphinema* (PV = 152) and *Pratylenchus* (PV = 72). Several of the nematode species associated with the two biofuels crops were plant parasites. Of these, *Pratylenchus penetrans*, *P. scribneri*, *P. crenatus*, *Helicotylenchus pseudorobustus*, *Hoplolaimus galeatus*, *X. americanum*, and *X. rivesi* are potentially the most damaging pests to *Miscanthus* × *giganteus* and switchgrass. Due to a lack of information, the damaging population thresholds of plant-parasitic nematodes to *Miscanthus* × *giganteus* and switchgrass are currently unknown. However, damage threshold value ranges have been reported for other monocotyledon hosts. If these damage threshold value ranges are any indication of the population densities required to impact *Miscanthus* × *giganteus* and switchgrass, then every state surveyed has potential for yield losses due to plant-parasitic nematodes. Specifically, *Helicotylenchus*, *Xiphinema*, *Pratylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconemella*, and *Longidorus* spp. were all found to have population densities within or above the threshold value ranges reported for other monocotyledon hosts.

Interest in the use of perennial grasses as energy crops in the United States and Europe has been increasing since the mid-1980s. The characteristics that make perennial grasses attractive for biomass production are their high yield potential, high contents of lignin and cellulose, and generally anticipated positive environmental impact. Two of the top energy-yielding cellulosic-ethanol feedstock plants are the perennial grasses *Miscanthus* × *giganteus* and switchgrass (*Panicum virgatum* L.) (19,45). *Miscanthus* × *giganteus*, a hybrid of *Miscanthus sinensis* and *M. sacchariflorus*, is a C₄ grass native to Asia. It has a high yield potential (19) and efficiently converts radiation to biomass (4). Switchgrass is a native prairie species adapted to most areas of the United States. It has been used for livestock feed, for wildlife habitat, and in soil conservation programs (32,45). These perennial grasses produce large quantities of biomass, require relatively little fertilizer and water, and can be used for fuel either by burning or in ethanol generation (45,46).

Research on the production of *Miscanthus* × *giganteus* and switchgrass as biofuel crops has mainly involved breeding for increased biomass yield and developing practices for nitrogen fertilization, weed control, and harvest. Few studies, however, have determined the impact of pests and diseases on *Miscanthus* × *giganteus* and switchgrass. Diseases and pests have the potential to cause significant constraints on plant growth and development, which puts the crops at risk for reductions in biomass yield and quality. Of the many pests and diseases, plant-parasitic nematodes

could be of great economic importance because they can directly reduce plant biomass and predispose plants to attack by other soil-borne pathogens (49,53).

Many species of plant-parasitic nematodes have been found associated with economically important monocot fiber and food crops throughout the world (28). Several of these reported in North America have been associated with monocotyledon hosts, including prairie grass, switchgrass, turf grass, and corn (6,11,13,24,42,56). The following genera of nematodes have been detected in the tallgrass prairie of the United States: *Filenchus*, *Helicotylenchus*, *Xiphinema*, *Aporcelaimellus*, *Dorylaimellus*, *Mesocriconema*, *Basiria*, *Plectus*, *Coslenchus*, and *Pungentus* (51). Twenty-one species of plant-parasitic nematodes belonging to the families *Aphelenchoidea*, *Tylenchinae*, *Psilenchinae*, and *Dorylaimoidea* were reported from the Kalsow Prairie in Iowa (41). In a survey of nematodes associated with established switchgrass stands in the south-central United States, Cassida et al. (6) detected *Xiphinema americanum*, *Tylenchorhynchus* spp., *Paratrichodorus minor*, *Criconemella ornata*, *Hoplolaimus magnistylus*, *Pratylenchus zeae*, *Helicotylenchus* spp., *Meloidogyne* spp., and *Paratylenchus* spp. These authors noted variation in the population density of plant-parasitic nematodes among switchgrass genotypes and, in some cases, nematode numbers were negatively correlated with stand persistence or dry matter yield (6). In a greenhouse study in Georgia, Johnson (23) reported that species of *Criconemella*, *Tylenchorhynchus*, and *Belonolaimus* readily reproduced on and reduced the biomass of six varieties of bermudagrass. In a similar study, Ingham and Detling (22) reported that nematodes in the orders Tylenchida and Dorylaimida consumed up to 16.4% of annual net root production of western wheatgrass and 8.9% of little bluestem grass in South Dakota. Sikora et al. (47) reported that a root-knot nematode, *Meloidogyne naasi*, alone or in combination with *P. penetrans* or *Tylenchorhynchus agri*, reduced the root growth and biomass of bentgrass in a greenhouse study. In contrast, Kokalis-

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Burelle (25) reported that switchgrass, when planted in rotation with peanut in Alabama, did not support root-knot nematodes but did support a large number of nonparasitic nematodes.

Few reports have documented the plant-parasitic nematodes associated with *Miscanthus sinensis*, a parent material of *Miscanthus × giganteus*. Root and rhizosphere soil samples taken in Japan included the nematodes *Nothocriconemella miscanthi* (35), *Verutus mesoangustus* (36), and *Radopholus sanoii* (37). *M. sinensis* has been reported to be a susceptible host of *Meloidogyne hapla* in Connecticut (27). Even fewer reports are available concerning nematodes associated with *Miscanthus × giganteus*. Mekete et al. (34) found *X. americanum*, *X. rivei*, and *Longidorus breviannulatus* associated with *Miscanthus × giganteus* in Illinois. *L. breviannulatus*, in particular, was associated with severe damage to the fibrous root system, including stunting and necrosis (34).

To help assess the importance of plant-parasitic nematodes in biofuels crops, a survey was conducted with the objective of providing preliminary information on the distribution, presence, and abundance of species associated with *Miscanthus × giganteus* and switchgrass.

Materials and Methods

Soil samples were collected from 24 *Miscanthus × giganteus* and 38 switchgrass managed plots in the states of Illinois, Iowa, and South Dakota during the 2008 growing season (Table 1). Additional soil samples were collected from 11 *Miscanthus × giganteus* and 10 switchgrass plots in the states of Illinois, Iowa, Kentucky, Tennessee, and Georgia during the 2009 growing season (Table 1). Samples were collected once per site in June through August of both years. All plots in Illinois, Iowa, and South Dakota were planted in 2002, 2005 and 2007, respectively, whereas plots in Kentucky, Tennessee, and Georgia were planted in 2008. All sampling sites, except Dudley Smith, IL, are university experimental plots. All plot sizes range from 100 to 200 m².

The elevation, latitude, and longitude for each sampling site were recorded using the Global Positioning System (GPS) (Garmin GPSMAP 60CSx; Garmin International Inc., KS). Geographical coordinates recorded for each farm were mapped with ArcView 3.2 (ESRI, Redlands, CA) (Fig. 1). Soil type was assigned using the GPS coordinates and the Natural Resource Conservation Service's

(NRCS) internet soil survey application (NRCS, Washington, DC.) (Table 1).

In each plot, 20 to 25 soil cores were collected near the rhizosphere of *Miscanthus × giganteus* and switchgrass plants at a depth of 20 to 30 cm. The cores were combined at the time of collection to form one composite soil sample per plot and placed in a plastic sample bag. All samples were kept at 4°C until processed, and nematodes were extracted within a 2-week period.

Composite samples were mixed thoroughly and one 100-cm³ subsample was used for nematode extraction via wet sieving followed by a sugar flotation centrifugal method (20). Briefly, 100 cm³ of soil was immersed in a bucket containing 2 liters of water and gently hand stirred until all soil aggregates disintegrated. After 30 s, the slurry was passed through nested 250-, 100-, and 25-μm pore sieves. This step was repeated until only sand and rocks remained in the bucket. Material on the 25-μm sieve was retained and processed by a sugar flotation centrifugal method to recover the nematodes collected. Larger nematodes in the genera of *Xiphinema*, *Longidorus*, and *Hoplolaimus* retained on the 100-μm sieve were collected and further processed using Baermann funnels that contained a single layer of cheesecloth (20). After 48 h, about 25 to 40 ml of water with nematodes was collected from the Baermann funnels. Nematodes were identified to the genus level with a dissecting microscope at ×40 to 63 and enumerated as the number of

Fig. 1. Distribution and Global Positioning System location of sampling sites. SD, South Dakota; IA, Iowa; IL, Illinois; KY, Kentucky; TN, Tennessee; GA, Georgia.

Table 1. Mean number of different plant-parasitic nematode genera extracted from 100-cm³ rhizosphere soil samples, collected from *Miscanthus × giganteus* and switchgrass plots in six midwestern and southeastern states in the United States^a

City, state	Year ^c	No. ^d	Soil type ^e	A Horizon (range, %)		Genera ^b										
				Clay	Sand	Helic	Xip	Praty	Long	Heter	Hop	Tylen	Cric	Paratri	Hemi	Paraty
Urbana, IL	Both	4	SCL	20–35	<15	284 (186)	360 (1,200)	206 (94)	0 (0)	66 (124)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	22 (8)
Savoy, IL	Both	2	SCL	20–35	<15	102 (138)	312 (108)	66 (162)	0 (0)	12 (72)	0 (0)	0 (0)	54 (26)	0 (0)	0 (0)	78 (132)
Dixon Springs, IL	2008	1	Silt loam	25–35	2–10	850 (386)	18 (50)	184 (96)	0 (0)	0 (0)	156 (224)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dudley Smith, IL	2008	1	Silt loam	20–27	<7	800 (260)	167 (246)	98 (26)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	52 (0)	0 (0)	0 (0)
Fairfield, IL	2008	1	Silt loam	20–27	<15	670 (660)	0 (0)	87 (24)	0 (0)	0 (0)	88 (0)	167 (80)	0 (0)	0 (0)	0 (0)	0 (0)
DeKalb, IL	2008	1	SCL	25–30	<10	147 (248)	80 (25)	90 (34)	0 (0)	18 (24)	0 (0)	0 (0)	0 (0)	0 (16)	0 (0)	0 (0)
Browntown, IL	2008	1	Silt loam	25–30	5–15	140 (660)	167 (120)	156 (88)	0 (0)	0 (0)	0 (0)	52 (146)	68 (126)	0 (0)	0 (0)	28 (67)
Havana, IL	2008	1	Sandy loam	5–18	50–80	130 (142)	0 (0)	175 (420)	26 (33)	26 (86)	88 (146)	24 (38)	160 (340)	0 (0)	0 (0)	12 (58)
Perry, IL	2008	1	Silt loam	18–27	<8	0 (0)	130 (660)	0 (0)	0 (0)	220 (48)	0 (0)	125 (32)	0 (0)	0 (0)	0 (0)	134 (220)
Ames , IA	Both	4	Clay loam	18–35	18–45	158 (690)	5 (16)	40 (112)	0 (0)	384 (647)	6 (32)	0 (0)	6 (22)	0 (0)	0 (20)	56 (126)
Brookings 1, SD	2008	2	SCL	27	17	– (380)	– (0)	– (28)	– (0)	– (68)	– (0)	– (286)	– (0)	– (0)	– (0)	– (96)
Brookings 2, SD	2008	1	Clay loam	28–35	30–40	– (87)	– (0)	– (0)	– (0)	– (46)	– (0)	– (127)	– (0)	– (0)	– (0)	– (132)
Bristol 1, SD	2008	3	Clay loam	35–45	20	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (12)
Bristol 2, SD	2008	1	Loam	20–30	>30	– (22)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (36)
Lexington, KY	2009	2	Silt loam	15–20	5–10	40 (320)	0 (0)	67 (53)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Milan, TN	2009	2	Silt loam	18–25	<15	– (480)	– (0)	– (10)	– (0)	– (4)	– (0)	– (0)	– (35)	– (0)	– (0)	– (0)
Midville, GA	2009	1	Loamy sand	1–10	70–85	96 (132)	0 (26)	27 (73)	5 (0)	0 (0)	0 (0)	187 (58)	0 (0)	0 (0)	0 (0)	0 (0)

^a Data presented here are averaged for 2008 and 2009 sampling periods.

^b *Helicotylenchus* (Helic), *Xiphinema* (Xip), *Pratylenchus* (Praty), *Longidorus* (Long), *Heterodera* (Heter), *Hoplolaimus* (Hop), *Tylenchorhynchus* (Tylen), *Criconebella* (Cric), *Paratrichodorus* (Paratri), *Hemicriconemoides* (Hemi), and *Paratylenchus* (Paraty). Counts in parentheses are mean numbers for *Miscanthus × giganteus* and switchgrass, respectively; 0 indicates zero count for the given nematode and – indicates no soil sample taken.

^c Sampling year; Both = 2008 and 2009.

^d Number of sites sampled.

^e SCL = silty clay loam. Data extracted from the United States Department of Agriculture, Natural Resource Conservation Service (<http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>) based on our survey Global Positioning System points.

nematodes per 100 cm³ of soil. Frequency of occurrence and population density were used to calculate a prominence value. Prominence value (PV) = population density × $\sqrt{\text{frequency of occurrence}/10}$ (10).

Identification of plant-parasitic nematodes was based on morphological methods. Some genera of nematodes were not identified to the species level because of a limited number of adult females. Nematodes were killed in hot water at 65°C and fixed in a formalin acetic acid fixative (4:1) solution containing 10 ml of formalin, 1 ml of glacial acetic acid, 2 ml of glycerol, and distilled water up to 100 ml (21). The nematode specimens were dehydrated using a glycerin method and mounted in pure glycerin on glass slides (20). Morphometric analyses of the nematode specimens were made using light microscopy and image-analyzing software (Zeiss Axion A1 imager version 4.0; New York). The species were identified with keys and descriptions by various authors (1,5,7,9,14–18,26,34).

Results

Plant-parasitic nematodes were found in all of the samples tested. The 11 dominant genera recovered from the soil samples were *Pratylenchus*, *Helicotylenchus*, *Xiphinema*, *Longidorus*, *Heterodera*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconebella*, *Paratrichodorus*, *Hemicriconebellides*, and *Paratylenchus* (Table 1). All of these genera contain one or more species of plant-parasitic

Table 2. Nematode damage threshold values, population density, and prominence values for the predominant nematode species associated with *Miscanthus × giganteus* (*M × g*) and switchgrass (Switch) plots in six midwestern and southeastern states in the United States in 2008 and 2009

Nematode genus	Damage ^b	Population ^c	Prominence value ^a	
			<i>M × g</i>	Switch
<i>Helicotylenchus</i>	300+	0–850	384	275
<i>Xiphinema</i>	50–100	0–1,200	131	152
<i>Pratylenchus</i>	50–100	0–420	70	72
<i>Hoplolaimus</i>	40–150	0–224	64	25
<i>Tylenchorhynchus</i>	150–300	0–226	24	32
<i>Criconebella</i>	300–600	0–340	8	20
<i>Longidorus</i>	5–25	0–54	3	2

^a Prominence value = population density × $\sqrt{\text{frequency of occurrence}/10}$.

^b Range (minimum–maximum) of damage threshold numbers per 100 cm³ of soil; guidelines used for monocotyledon hosts based on findings from the University of Illinois (2,3).

^c Range (minimum–maximum) of population density per 100 cm³ of rhizosphere soil collected from *Miscanthus × giganteus* and switchgrass plots. Population densities are pooled data showing range of surveyed nematode densities per 100 cm³ rhizosphere soil collected from *Miscanthus × giganteus* and switchgrass plots.

nematodes capable of causing significant biomass reductions on monocotyledonous hosts. The results show variability in the population levels of plant-parasitic nematodes among sampling sites (Table 1). At most locations, data on the frequency of occurrence and population densities for the different groups were found above damage threshold levels established for monocotyledon hosts by the University of Illinois (Table 2) (2,3).

This survey indicates that *Helicotylenchus*, *Xiphinema*, and *Pratylenchus* spp. are widespread, have a high prominence value, and are above the damage thresholds established for some monocotyledon hosts in approximately one-third of the samples tested (Figs. 2 and 3; Table 2). *Helicotylenchus* spp. were common and recorded at 90.5 and 91.6% in *Miscanthus × giganteus* and switchgrass samples, respectively. The high abundance and frequency indicate that species of this genus most likely have a host-parasite relationship with *Miscanthus × giganteus* and switchgrass. *Xiphinema* spp. were found in 83.8 and 75.0% of the *Miscanthus × giganteus* and switchgrass samples, respectively. The highest number recorded in the survey (1,200 per 100 cm³ of soil) was observed at the Urbana, IL, sampling site and suggests that *Xiphinema* spp. may have a host-parasite relationship with *Miscanthus × giganteus* and switchgrass. *Pratylenchus* spp. were found 91.9 and 83.3% of the *Miscanthus × giganteus* and switchgrass samples, respectively. The highest population densities of *Pratylenchus* spp. were recorded from three of the sites in Illinois: Dixon Springs (184 per 100 cm³ of soil), Urbana (206 per 100 cm³ of soil), and Havana (420 per 100 cm³ of soil). Population levels of *L. breviannulatus* were high (33 per 100 cm³ of soil) at the Havana, IL sampling site and caused visible stunting of lateral roots and destruction of the fibrous root system (28).

Hoplolaimus, *Heterodera*, *Tylenchorhynchus*, *Criconebella*, *Paratrichodorus*, and *Hemicriconebellides* spp. were identified at several sampling sites (Table 1). When these species were present, they had relatively high population numbers but were not ubiquitous. *Paratylenchus* spp. were recorded in 43.2 and 33.3% of sites sampled in *Miscanthus × giganteus* and switchgrass samples, respectively. This genera of nematode is frequently identified in association with several plant species, though their potential for direct damage to crops is not well known.

Concomitant infestations by *Pratylenchus*, *Xiphinema*, *Longidorus*, and *Hoplolaimus* spp. were observed, and 10% of the sampling sites were co-infected by more than one of these nematodes. For example, *Longidorus* spp. were detected concomitantly with *Xiphinema*, *Pratylenchus*, and *Hoplolaimus* spp. at the Havana, IL sampling site. Population levels in most of the concomitantly infested fields were above the established damage threshold levels established for a monocotyledon host (Table 2).

Fig. 2. Frequency (%) and abundance of the major nematode species from rhizosphere soil of *Miscanthus × giganteus* at 12 field sites in the midwestern and southeastern United States during 2008 and 2009 soil surveys.

Soil types of sampling fields varied in texture with sandy loam, silty loam, silty clay loam, clay loam, loam, and loamy sand (Table 1). Soil pH ranged from 5.6 to 8.8. There was no consistent trend in numbers of individual or combined nematode population densities in relation to soil type and soil pH except for *L. breviannulatus*. The occurrence of this nematode seems to be localized and restricted to highly sandy soils in Havana, IL (Table 1).

Our survey results from 2008 and 2009 sampling season identified, to the species level, 20 and 24 plant-parasitic nematodes associated with *Miscanthus × giganteus* and switchgrass, respectively (Tables 3 and 4). The species that are potentially damaging to these biofuel crops include six species of *Pratylenchus* (*P. penetrans*, *P. hexincisus*, *P. scribneri*, *P. brachyurus*, *P. crenatus*, and *P. neglectus*), two species of *Xiphinema* (*X. rivesi* and *X. americanum*), three species of *Helicotylenchus* (*Helicotylenchus pseudorobustus*, *H. platyurus*, and *H. digonicus*) two species of *Longidorus* (*L. breviannulatus* and *L. americanum*), *Hoplolaimus galeatus*, *Heterodera glycines*, and two unidentified populations of *Heterodera*.

Discussion

The objective of this study was to determine population densities and the extent of infestation by plant-parasitic nematodes on *Miscanthus × giganteus* and switchgrass in experimental plots in Illinois, Iowa, South Dakota, Kentucky, Tennessee, and Georgia of the United States. Our survey results demonstrate the widespread occurrence of plant-parasitic nematodes in more than 90% of *Miscanthus × giganteus* and switchgrass plots sampled during 2008 and 2009. Comparison of these results with other surveys on switchgrass indicates the presence of a similar composition of plant-parasitic nematodes of reported significance (6,14). All sites sampled had at least two nematode species that have been reported to reduce biomass in most monocotyledons hosts (2,3).

The damage potential of any plant-parasitic nematode species to a given plant species depends on the host plant susceptibility, population density, and other ecological factors such as soil texture, temperature, moisture, organic matter, pH, and associated soil microorganisms (33,38). *Pratylenchus*, *Xiphinema*, *Longidorus*, *Hoplolaimus*, *Tylenchorhynchus*, *Helicotylenchus*, and *Cricone-mella* spp. have been reported to cause direct and indirect damage to several monocotyledons such as corn, bent grass, switchgrass, and turf grasses (6,22,28,33,34,49). These groups of nematodes are probably of the most concern to *Miscanthus × giganteus* and switchgrass producers, because they have shown the most damage elsewhere. Though population levels of these nematodes that damage *Miscanthus × giganteus* and switchgrass are not known, the nematode densities encountered in our survey may represent the potential for disease in biofuel production based on the damage

threshold densities reported for nematodes on other monocotyledon hosts such as corn (2,3).

Genus *Helicotylenchus* was the most frequently identified nematode in our survey. High population densities of this genus can cause root damage and establish entry points for other soilborne pathogens (55). Some studies have suggested high population densities (>300 nematodes per 100 cm³ of soil) of *Helicotylenchus* spp. are necessary to cause economic damage to hosts (2,3). However, a report from Iowa suggests that *Helicotylenchus pseudorobustus* may cause damage to corn and soybeans when population densities are 100 nematodes per 100 cm³ of soil (40). *H. pseudorobustus* was the primary species found during this survey. It was identified at most of the sampling sites on both *Miscanthus × giganteus* and switchgrass, and it has been associated with a wide range of plants, including prairie grasses, legumes, and corn (12,31,41,50,55). Damage attributed to *H. pseudorobustus* has been reported in native prairie grasses, turf grasses (8,52), and soybean

Table 3. Plant-parasitic nematodes species identified from the rhizosphere of 35 *Miscanthus × giganteus* plots at 12 field sites in the midwestern and southeastern United States during 2008 and 2009 rhizosphere soil surveys

Nematode	Sample sites (no. of plots surveyed) ^a			
	IL (29)	IA (2)	KY (2)	GA (2)
<i>Dorylaimida</i>				
<i>Xiphinema americanum</i>	+	–	–	+
<i>X. rivesi</i>	+	–	–	–
<i>Xiphinema</i> spp.	–	–	–	–
<i>Longidorus breviannulatus</i>	+	–	–	–
<i>Longidorus</i> sp.	–	–	–	+
<i>Triplonchida</i>				
<i>Paratrichodorus</i> sp.	+	–	–	–
<i>Tylenchida</i>				
<i>Pratylenchus penetrans</i>	+	–	–	–
<i>P. hexincisus</i>	+	–	+	+
<i>P. scribneri</i>	+	+	+	+
<i>P. crenatus</i>	+	+	–	–
<i>Hoplolaimus galeatus</i>	+	+	+	–
<i>Heterodera glycines</i>	+	+	+	–
<i>Helicotylenchus pseudorobustus</i>	+	+	–	–
<i>H. digonicus</i>	+	–	+	–
<i>H. platyurus</i>	–	–	–	–
<i>Helicotylenchus</i> spp.	+	–	–	+
<i>Tylenchorhynchus agri</i>	–	–	–	–
<i>Tylenchorhynchus</i> sp.	+	–	+	–
<i>Cricone-mella</i> spp.	+	–	–	–
<i>Paratylenchus</i> sp.	+	–	–	–

^a IL = Illinois, IA = Iowa, KY = Kentucky, and GA = Georgia; + indicates positive detection and – indicates negative detection.

Fig. 3. Frequency (%) and abundance of the major nematode species from rhizosphere soil of switchgrass plots at 17 field sites in the midwestern and southeastern United States during 2008 and 2009 soil surveys.

(31). Accurate estimates of the damage threshold of *H. pseudorobustus* are needed. Populations counted in this survey exceeded the aforementioned thresholds in both *Miscanthus* × *giganteus* and switchgrass plots. Further studies are necessary to determine the economic importance of this species to *Miscanthus* × *giganteus* and switchgrass.

Our survey data showed the presence of two species of *Xiphinema*: *X. americanum* and *X. rivesi*. The highest population density (1,200 per 100 cm³ of soil) was recorded for *X. americanum* at the plots near Urbana, IL. The *Xiphinema* nematodes are commonly found in North America and have a wide host range that includes grasses, soybean, and perennial orchards (12,41,43,54). They are ectoparasites and known vectors of certain viruses (40). The impact of *Xiphinema* spp. on *Miscanthus* × *giganteus* and switchgrass has not been established. Future work is necessary to determine their importance to *Miscanthus* × *giganteus* and switchgrass.

Six species of *Pratylenchus* (*P. penetrans*, *P. hexincisus*, *P. scribneri*, *P. brachyurus*, *P. crenatus*, and *P. neglectus*) were identified in our survey. The root-lesion nematodes *P. scribneri*, *P. penetrans*, and *P. crenatus* were quite common and frequently detected at several *Miscanthus* × *giganteus* and switchgrass sampling sites. These species have been reported as pests of several plant species such as grasses, cereals, and legumes and, thus, they could be considered potential pests of *Miscanthus* × *giganteus* and switchgrass (6,10,12,13,40). When compared with damage thresholds for monocotyledon hosts, the results of this study suggest that *Pratylenchus* species will be a potential problem for *Miscanthus* × *giganteus* and switchgrass production.

In our survey, *L. breviannulatus* was recovered from the Havana, IL sampling site at high population densities, and they appeared to be stunting the lateral roots and destroying the fibrous roots of *Miscanthus* × *giganteus*. These observations indicate that this nematode may have the potential to substantially reduce biomass production. First described from Iowa and associated with corn (39), this species has been found in North America in association with several plant hosts such as corn and several grass species (29,30,34,48). Malek et al. (30) considered *L. breviannulatus* to be

the most devastating nematode attacking corn. The relationship between nematode density and *Miscanthus* × *giganteus* and switchgrass yield remains unclear and must be studied further.

Detections of different species from the genera *Hoplolaimus*, *Heterodera*, *Tylenchorhynchus*, *Criconemella*, *Paratrichodorus*, and *Hemicriconemoides* revealed low frequencies in the sampled *Miscanthus* × *giganteus* and switchgrass plots. It is probable that a host-parasite relationship exists, although there does not seem to be a strong parasitic relationship with *Miscanthus* × *giganteus* and switchgrass. However, it would be prudent to monitor these fields closely to determine whether a nematode population increase occurs, which could indicate a possible host-parasite relationship.

To our knowledge, this is the first detailed report and description of these plant-parasitic nematodes associated with *Miscanthus* × *giganteus*. Further studies have been initiated to determine the host suitability and damage thresholds of *Pratylenchus* and *Longidorus* spp. to *Miscanthus* × *giganteus* and switchgrass under greenhouse and field conditions. Future work will focus on host suitability, damage thresholds, and population dynamics of the most prevalent nematodes associated with *Miscanthus* × *giganteus* and switchgrass. This information will be used in estimating the impact of nematodes on the yield potential of the *Miscanthus* × *giganteus* and switchgrass biomass.

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Table 4. Plant-parasitic nematodes species identified from the rhizosphere of 48 switchgrass plots at 17 field sites in the midwestern and southeastern United States during 2008 and 2009 rhizosphere soil surveys

Nematode	Sample sites (no. of plots surveyed) ^a					
	IL (29)	IA (2)	SD (11)	KY (2)	TN (2)	GA (2)
<i>Dorylaimida</i>						
<i>Xiphinema americanum</i>	+	+	—	+	—	+
<i>X. rivesi</i>	+	—	—	—	—	—
<i>Xiphinema</i> spp.	—	—	—	—	+	—
<i>Longidorus breviannulatus</i>	+	—	—	—	—	—
<i>Longidorus</i> sp.	—	—	—	—	—	+
<i>Triplonchida</i>						
<i>Paratrichodorus</i> sp.	+	—	—	—	+	+
<i>Tylenchida</i>						
<i>Pratylenchus penetrans</i>	+	—	+	—	—	—
<i>P. hexincisus</i>	+	—	—	+	—	+
<i>P. scribneri</i>	+	+	—	+	+	+
<i>P. brachyurus</i>	—	—	+	—	—	—
<i>P. crenatus</i>	+	+	—	—	—	—
<i>P. neglectus</i>	+	—	—	—	—	—
<i>Hoplolaimus galeatus</i>	+	+	—	+	+	—
<i>Heterodera glycines</i>	+	+	—	+	+	—
<i>Heterodera</i> sp.	+	—	—	—	—	—
<i>Helicotylenchus pseudorobustus</i>	+	+	+	—	+	—
<i>H. digonicus</i>	+	—	—	+	—	—
<i>H. platyurus</i>	—	—	+	—	—	—
<i>Helicotylenchus</i> spp.	+	—	—	—	—	+
<i>Tylenchorhynchus nudus</i>	—	—	+	—	—	—
<i>T. agri</i>	+	—	—	—	—	—
<i>Tylenchorhynchus</i> sp.	+	—	—	+	—	—
<i>Criconemella</i> spp.	+	+	—	+	+	—
<i>Paratylenchus</i> sp.	+	—	—	—	—	—

^a IL = Illinois, IA = Iowa, SD = South Dakota, KY = Kentucky, TN = Tennessee, and GA = Georgia; + indicates positive detection and — indicates negative detection.

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